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Biochemical genetic traits related to malaria in the Alorese: Another advantage to hepatitis B virus?

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ABSTRAK

Abdul Salam M. Sofro – *Trait genetik biokimia yang berkaitan dengan malaria pada penduduk P. Alor: keuntungan lain terhadap virus hepatitis–B?*

Meskipun secara geografis, budaya dan antropologi ragawi orang Alor berhubungan erat dengan penduduk Nusa Tenggara Timur lainnya yang berada dalam klaster Indonesia timur, tetapi dengan analisis jarak genetik mereka berada sedikit di luar. Namun demikian secara umum populasi Indonesia khususnya populasi di Nusa Tenggara telah lama terpapar faktor seleksi alam yang kurang lebih sama yaitu malaria. Di samping itu, kawasan ini dikenal memiliki prevalensi infeksi virus hepatitis B (HBV) cukup tinggi.

Penelitian ini dimaksudkan untuk melihat trait genetika biokimia yang mencirikan pola klinal populasi di Indonesia serta yang barangkali menawarkan keunggulan selektif terhadap malaria yang merupakan salah satu faktor seleksi alam utama di daerah Alor. Di samping itu juga akan dipelajari kemungkinan antaraksi trait genetik tersebut dengan hepatitis.

Hasil pemeriksaan sistem golongan darah ABO menunjukkan tingginya frekuensi golongan darah O (49,18%), sedangkan A dan B masing-masing 24,59% dan 23,77%. Hasil ini agak berbeda dengan distribusi di Yogyakarta dengan populasi yang lebih Mongolid dengan frekuensi golongan darah B agak tinggi (29,23%) sebagaimana kebanyakan populasi Asia Tenggara. Dari tiga trait yang terkait dengan malaria, tidak satupun HbE atau pengemban thalassemia-ß ditemukan dari 122 subjek yang diperiksa. Namun demikian, kekurangan *glucose-6-phosphate dehydrogenase* (G6PD) ditemukan pada 8 subjek (6,6%) dan ovalositosis pada 15 subjek (12.3%). Dari pemeriksaan HBsAg yang dapat dilakukan pada 109 subjek, didapatkan 13 subjek dengan HBsAg positif. Yang menarik, dari 7 subjek dengan kekurangan G6PD hanya satu subjek menunjukkan HBsAg positif, sementara tidak satupun dari 8 subjek ovalositosis menunjukkan HbsAg positif. Meskipun secara statistik tidak bermakna, kemungkinan bahwa gena mutan khususnya untuk ovalositosis memiliki keunggulan selektif terhadap infeksi hepatitis perlu dipikirkan. Mungkin saja bahwa gena mutan diekspresikan di sel hati yang menyebabkan gangguan adsorbsi dan penetrasi virus ke dalam sel hati. Diperlukan penelitian lebih lanjut untuk dapat menjelaskan fenomena yang menarik ini.

Key words : biochemical genetic trait – glucose-6-phosphatedehydrogenase deficiency – ovalositosis–β thalassemia

(Berkala Ilmu Kedokteran Vol. 27, No. 2, Juni 1995)

INTRODUCTION

Despite its geographical, cultural and anthropological affinities, employing the genetic distance analysis, the Alorese and the Savunese stand a little apart from the other eastern Lesser Sunda or Nusa Tenggara population.[1] This surprising result rendered to some speculations. Apart from their small sample size, the local legend among the Savunese concerning with the influence of Javanese Hindu Kingdom might interfere their genetic constitution. This may not be so for the Alorese. It is suggested that the Alorese is one of the Indonesian population that still preserve the remnant Negrito genes. In addition, the hemoglobin E (HbE) gene frequency

in the Savunese is among the highest (25%) in the country, while not even single HbE was found in the Alorese.[2] Being Southeast Asian genetic marker, the high HbE gene frequency in the Savunese and its absence in the Alorese to some extend reveal some degrees of difference of Southeast Asian genes influence to those population.

Coincidentally, the two population are among those eastern Nusa Tenggara population living in a malaria endemic environment. In fact, it has long been suggested that some genetic factors might confer selective advantage in such a particular environment. In this respect, HbE variant which is included in the thalassemia group coexists with the distribution of ovalocytosis and glucose-6-phosphate dehydrogenase (G6PD) deficiency.[3] Unfortunately the previous work was carried out in a limited number of subjects.[4] Therefore, studying biochemical genetic markers, particularly those related to malaria in the Alorese with a larger series of samples, will provide a more reliable figure that can be used for further studies.

In addition to malaria, it is worth to put forward the possible interaction of some other infectious agents with particular biochemical genetic traits. In this case, being quite prevalent in the region, hepatitis due to hepatitis B virus (HBV) would be a suitable subject to be considered. So far, prevalence of this particular viral disease in the Alorese is not available. Whether it is also prevalent in the Alorese and shows any interaction with the above mentioned traits is a matter of conjecture. The present study tries to reveal the distribution of some biochemical genetic traits related to malaria and clarify their possible interactions with HBV.

MATERIALS AND METHODS

Study location and subjects

The field study was carried out in Alor island, the largest island in the Alor area, East Nusa Tenggara Province (FIGURE 1). The subjects were, healthy adult unrelated individuals of Alor origin. They consisted of 57 male and 65 female students of SMA Negeri and SMEA Negeri Kalabahi, Alor who voluntarily participated in the study.

About 10-15 ml of blood were drawn from antecubital vein using EDTA-anticoagulated Terumo venoject vacutainer. All these specimens were brought to the IUC-Biotechnology

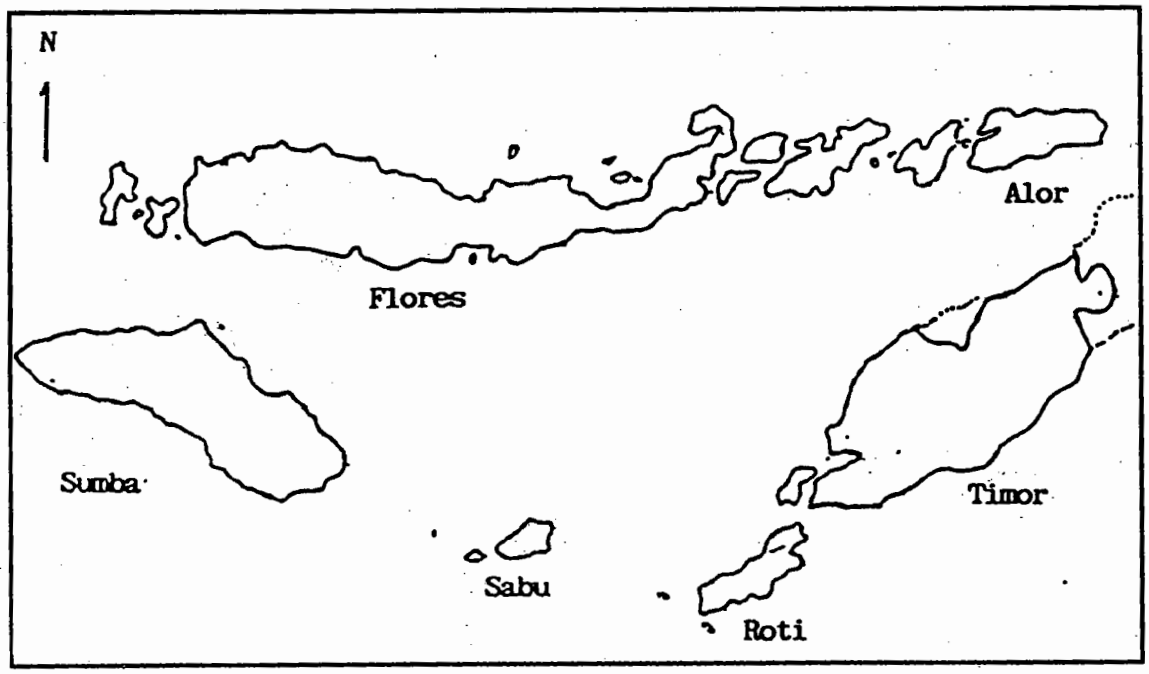

FIGURE 1. – Map of East Nusa Tenggara showing the study location

laboratory until further examination. Blood smears were prepared right after blood drawing from the tip of the venoject needles.

Laboratory examination

Within five days after the collection, the blood was examined for hemoglobin level, packed cell volume, HbA_2 concentration, the ABO blood groups, hepatitis B, and glucose-6-phosphate dehydrogenase (G6PD) activity. In addition, ovalocytosis was examined from the blood smear.

Cyanmethemoglobin was spectrophotometrically measured to determine the hemoglobin (Hb) level. Microcolumn chromatography from Helena was used to measure HbA_2 concentration and identify HbE. The ABO blood group was serologically determined, while fluorescence spot test employing test kit from Sigma cat. no. 202 was used to examine G6PD activity. Hepatitis B surface antigen (HBsAg) was determined by micro ELISA method using Hepanostika HBsAg UniForm II test kit from Organon.

RESULTS AND DISCUSSION

Description of study location and population

Alor district is one of 12 districts in the East Nusa Tenggara Province. It consists of islands, nine of which are inhabited. It is located between 123 48' and 125 8' east longitude and 8 6' and 8 16' south lattitude. Within an area of 2,864.64 square km and 147,678 population, the population density is 52 per square km. Due to some degrees of isolation, more than 25 languages or dialects are practised in the district. Some of the inhabitants live in the hilly parts of the island and seem to preserve their traditional life style. To overcome the language barrier, the majority speak Indonesian. Endogamous mating is a common practice, with a few exception for those living in the coastal areas. Physically, they have some degrees of Melanesian characteristics that fall into the eastern Indonesian cluster.[1]

Blood indices

Results of routine blood indices are shown in TABLE 1. Hemoglobin concentration either in male or female subjects appeared to be normal. It ranges from 11.00 to 20.55 g/dl with mean value of 15.82 ± 2.60 g/dl in male and from 8.92 to 18.7 g/dl in female with the mean value of 13.54 ± 2.18 g/dl. Packed cell volume was also normal with mean value of 48.35 ± 8.82 % (32-68%) for male and 41.53 ± 7.14 % (25-58%) for female. Normal ranges were also observed for RBC, MCV and MCHC.

In general, apart from a few subjects with exeptionally low hemoglobin concentration, subjects of the present study were hematologically normal. This was consistent with their apparently healthy physical appearance as required for this study.

ABO blood group

Among biochemical genetic traits examined was the ABO blood group system. This was the first polymorphic system to be discovered and the broad pattern of the gene frequencies of the

TABLE 1. – Hemoglobin (Hb) concentration (g/dl), packed cell volume (PCV, %), red blood cell count (RBC, million/cu mm), mean corpuscular volume (MCV, fl), and mean corpuscular hemoglobin concentration (MCHC, %) in the Alorese (mean ± SD and range).

Blood indices	Male (n = 57)	Female (n = 65)
Hb (g/dl)	15.82 ± 2.60 (20.55 – 11.00)	13.54 ± 2.18 (18.70 – 8.92)
PCV (%)	48.35 ± 8.82 (32 – 68)	41.53 ± 7.14 (25 – 58)
RBC (10^6/cu mm)	5.41 ± 1.07 (3.58 – 8.29)	4.67 ± 0.83 (3.02 – 6.93)
MCV (fl)	89.85 ± 6.92 (65.10 – 99.03)	89.13 ± 7.81 (73.27 – 106.12)
MCHC (%)	32.94 ± 2.87 (26.85 – 43.08)	32.84 ± 2.96 (22.89 – 40.08)

alleles A, B and O throughout the world had been available. The allele frequencies vary from one population to another.

The distribution of this blood group in the present study is shown in TABLE 2. The A and B blood group are almost equal, i.e 24.59% and 23.77% respectively, while AB is only 2.46% and O being the highest (49.18%). Using the Bernstein' formula, the gene frequencies were p = 1 - 0.4918 + 0.2377 = 0.1459 for A; q = 1- 0.4918 + 0.2459 = 0.1411 for B and r = 0.4918 = 0.7013 for O respectively. Thus $p + q + r$ = 0.1459 + 0.1411 + 0.7013 = 0.9883 which at first glance agreed well to the expectation i.e. 1. With the Xi = 1.171 [$Xi = 2n(1 + r/pq)D$ and $D = 1-(p+q+r)$], it is confirmed that the value found was in good agreement with the Hardy-Weinberg expectation. The gene frequency for A was similar to that for B (14.59% and 14.11%), while gene frequency for O being the highest. These gene frequencies might be compared with those from Yogyakarta. Analysing data from 22,593 blood donors in Yogyakarta, Sofro (unpublished) found the blood group frequencies for A, B, AB and O were 23.83%, 29.23%, 9.19% and 37.75% respectively. From these figures the gene frequencies for A, B and O were 0.1816, 0.2153, and 0.6144 respectively. It was apparent that either the gene frequency for A or B were higher in Yogyakarta, than those in the Alorese. In contrast, the gene frequency for O was lower in Yogyakarta than that in the Alorese.

Distribution of the ABO blood group in those two population is shown in FIGURE 2. The higher gene frequency of B in Yogyakarta was comparable with most of South East Asian population which in general were characterized by a high gene frequency of B, a Mongoloid characteristic[5]. Since the Alorese is known to have strong Melanesian influence, it is reasonable

TABLE 2. – Distribution of the ABO blood group phenotypes in the Alorese

Sex	Phenotypes			
	A	B	AB	O
Male (n = 57)	19	11	1	26
Female (n = 65)	11	18	2	34
Total (n = 122)	30 (24.59%)	29 (23.77%)	3 (2.46%)	60 (49.18%)

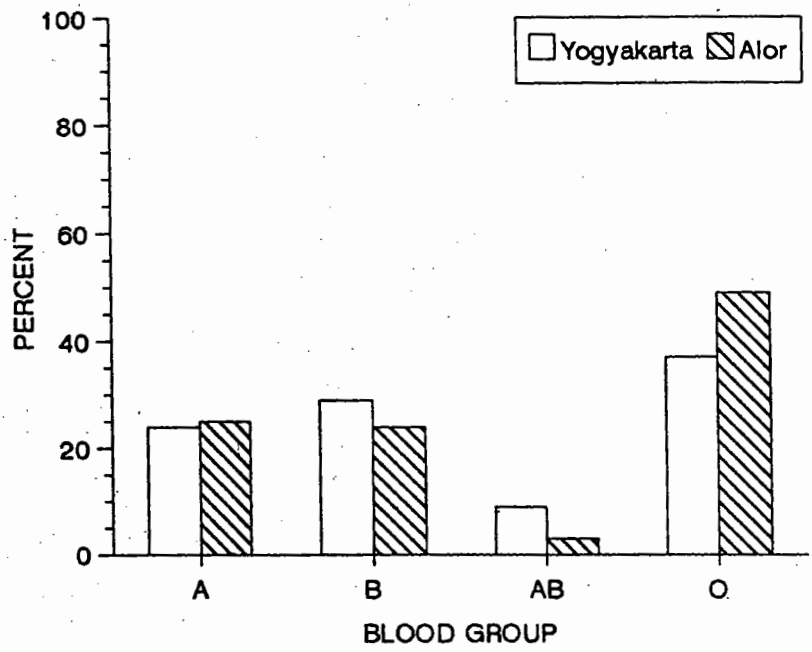

FIGURE 2. – Distribution of ABO blood group in Yogyakarta and Alor

that the frequency of B is lower than that in Yogyakarta.

It has long been known that polymorphism of this system is present in almost all human population. Some selective factors influencing the distribution of the ABO phenoptypes have been thoroughly discussed.[6] However, worldwide distribution of the ABO polymorphism suggests that tropical diseases are unlikely to play a role in selection. Instead, various other factors might work together to maintain this polymorphism. Bearing all these in mind, in addition to the genetic relationship of the Indonesian population,[1] the present distribution of the ABO phenotypes in the Alorese may reflect the result of population history and gene diffusion.

Genetic traits related to malaria

Distribution of three genetic traits related to malaria is shown in TABLE 3. Previous study showed that these traits were widely distributed in Indonesia.[3,4] Based on the population study, an inverse relationship was observed between the distribution of G6PD deficiency and ovalocytosis. At that time no data was available on the distribution of thalassemia. However, hemoglobin E (HbE), a structural variant of hemoglobin related to ß-thalassemia was distributed in the series of samples in the present study, the absence of HbE may represent somewhat weak penetration of this South East Asian gene into the population. This suggestion was also supported by the absence of Tf^{DChi}, a Mongoloid gene marker in this respective population.[8] In contrast, G6PD deficiency and ovalocytosis are common.

Among 122 individuals examined, eight individuals (6.6%) were G6PD deficient with equal frequency in male and female. Regardless the underlying molecular defect of this trait, the occurence of G6PD deficiency in this study was consistent with its occurence in the related neighbouring population with similar malaria condition. In fact, as one of the most common enzyme genetic disorders, G6PD deficiency was very heterogenous. At present more than 400 variants have been reported.[9] Molecular variation underlying this biochemical defect renders their phenotypic expression. Some of their molecular defects had been elucidated but no report is available from Indonesia. Considering the long history of human occupation in the region with various factors influencing their gene pools, it would not be surprising if in addition to the common variant, some rare variants might be found in Indonesian population. Examples can be observed in other genetic loci.[8,10] With malaria as the most prominent disease in the region, the common variant confering selective advantage would tend to increase in frequency.

TABLE 3. – Glucose-6-phosphate dehydrogenase (G6PD) deficiency, ovalocytosis and ß-thalassemia/HbE in the Alorese

Trait	Male (n = 57)	Female (n = 65)	Total (n = 122)
G6PD deficiency	4 (3.3%)	4 (3.3%)	8 (6.6%)
Ovalocytosis	4 (3.3%)	11 (9.0%)	15 (12.3%)
β-Thal or HbE	0 (0%)	0 (0%)	0 (0%)

archipelago. During the present study, either ß-thalassemia or HbE was not found. In the previous study, despite the presence of HbE in the neighbouring population, its absence in the Alorese was understandable possibly due to their small sample size.[2] This phenomenon resembled that in Flores with population that was genetically related where HbE was very low, but ß-thalassemia carrier was reasonably high.[7] With a larger

Along with G6PD deficiency, ovalocytosis is also found in 15 subjects (12.3%). This figure is a little higher compared to the previous study, in which ovalocytosis was found with frequencies range from 0.2 % to 23.7%.[4] Despite its wide distribution in Indonesia, no detail studies concerning the molecular genetic defect had been

reported until quite recently[11]. This trait did not seem to be clinically harmful, but the distribution in various population was of special interest. Some evidences suggested that this so-called Southeast Asian Ovalocytosis (SAO) is resistant to merozoite invasion.[12] The most recent study in Jakarta also confirmed this suggestion.[13]

In addition to the above biochemical genetic traits, subjects were screened for HBsAg. Since hemolysis occured in some samples, HBsAg could only be determined in 109 subjects from which 13 (12%) were HBsAg positive. This reasonably high figure was comparable to those reported in other Nusa Tenggara population especially in Lombok which was the highest.

As shown in TABLE 4 and TABLE 5, the expected interaction of HBsAg could be observed with either G6PD deficiency or ovalocytosis. TABLE 4 shows that 12 HBsAg positive subjects were found among 102 normal subjects, compared with only one HBsAg positive among seven G6PD deficient subjects. Almost similarly, TABLE 5 shows that 13 HBsAg positive subjects were found in 101 normal red cells subjects, whereas not even single HBsAg positif was found in eight ovalocytic individuals. However, employing the X^2 test for 2x2 table the result did not confirm statistical significance. Indeed, observation based on a population study, particularly the prevalence of any infectious disease in a population with certain genetic traits required various considerations. In such studies, high frequency of genetic traits in some population was important. So far, only limited population with high frequency of G6PD deficiency or ovalocytosis are available in which the prevalence of HBsAg positive is high. The fact that HBsAg negative were observed in majority of G6PD deficient subjects might not imply that the trait confer advantage to the HBV infection. It is very likely that G6PD deficiency has nothing to do with adsorption of the virus onto and their replication within the liver cells. However, the observation that ovalocytic individuals in this study seem to be free from HBV needs further studies.

In this case, assuming that the mutant gene for all ovalocytosis found in Indonesia were identical,[11,13] the Alorese with ovalocytosis should possess 27 nucleotides deletion coding for nine amino acids in their band-3 protein of their erythrocyte membrane. This band-3 protein was an anion-exchange protein which composed of N-terminal cytoplasmic domain and a membrane domain. The latter spanned the bilayer 14 times that was responsible for anion-exchange activity. The deleted segment represented the interface between the N-terminal cytoplasmic part of the protein and the first transmembrane segment. The biophysical consequences of this mutation were

TABLE 4. – Hepatitis B virus (HBV) infection in the Alorese with normal and G6PD deficiency

	HBsAg (+)	HBsAg (–)	Total
Normal	12	90	102
G6PD deficiency	1	6	7
Total	13	96	109

$\chi_1^2 = 0.0422$ $p>0.05$

TABLE 5. – Hepatitis B virus (HBV) infection in the Alorese with normal erythrocytes and ovalocytosis

	HBsAg (+)	HBsAg (–)	Total
Normal	13	88	101
Ovalocytosis	0	8	8
Total	13	96	109

$\chi_1^2 = 1.1630$ $p>0.05$

marked decrease in lateral mobility of band 3 in the membrane and an increase in membrane extentional rigidity.[12] It followed that this mutation was responsible for the resistance of ovalocytosis to malaria parasite invasion.

The next question was how this mutant might serve as a barrier for HBV infection. From the genetic point of view, any somatic cells should bear this 27 nucleotides deletion. The only problem was whether the defective gene was expressed to produce an anomaly of the membrane structure resembling that in erythrocytes. It was possible that the mutant gene was not expressed in the liver cells due to different and special function of their membrane. However, it was also equally possible that the mutant gene to some extent was expressed, so that the band-3 protein would constitute part of the overall liver cell membrane. If this was the case, similar biophysical consequences in ovalocytosis might also occur in the liver cells, since HBV had to be absorbed onto the liver cell membrane.[14] Alteration in the membrane structure might alter the affinity of the HBV to the membrane which in turn also prevented its penetration into the cells. Once HBV penetrated the cell membrane and eventually the nuclear membrane, HBV-DNA would be integrated in the host DNA.

Several studies have been conducted to examine the integration of HBV-DNA in the liver cells either in chronic liver diseases or tumor patients as well as human cell lines.[15,16,17] It was shown in their previous study[15] that HBV-DNA existed in tumor from all patients whose serum was HBsAg positive and was absent in tumor from patients whose serum was anti-HBsAg positive. It was suggested that integration of HBV-DNA occurred in nontumorous liver tissue preceded the development of gross neoplasia. The penetration into the cell membrane was the crucial step for HBV to replicate further and produce HBsAg. Since it was also shown that HBsAg was translated from free and integrated viral DNA in the liver,[18] then HBsAg positive serum in normal individuals might reflect the presence of HBV-DNA in liver cells either in free or integrated conditions. In this case, HBsAg positive individuals during the present study might have had HBV-DNA integrated in their liver cells. On the other hand, those with HBsAg negative might not have had HBV-DNA in their liver cells. Of course, it would be impossible to examine the liver cells of those ovalocytic individuals with HBsAg negative serum. Whether they were really free from HBV and were the underlying mechanisms were uneasy to describe. The only fact was that none of the ovalocytic individuals was HBsAg positive. Some other approaches should be explored to clarify this speculation.

CONCLUSION

Examination of three blood genetic traits related to malaria revealed that HbE or ß-thalassemia commonly found in Indonesian populations was surprisingly absent in the Alorese. The absence of HbE or ß-thalassemia might represent somewhat weak penetration of this South East Asian gene into this population which is supported in part by relatively low B blood group and distribution of some genetic markers previously studied.

In contrast, reasonable frequency of G6PD deficiency and ovalocytosis confirmed the results of previous studies. In addition, 13 HBsAg positive are found among 109 individuals screened. Despite statistically insignificant, the absence of HBsAg positive among ovalocytic individuals suggested the possibility that this genetic trait might confer selective advantage to HBV infection. Studies at the population level using populations with higher frequencies of such genetic trait and HBV infection was almost impossible. Therefore, studies at the cellular and molecular levels are still required to clarify whether the ovalocytosis mutant is expressed in the liver which in turn prevents the adsorption and replication of the HBV in the liver cells.

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